Graft-Versus-Host Reaction in Small Bowel Transplantation and Possibilities for its Circumvention

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Investigations in the field of small bowel transplantation have been carried out since 1959 in various experimental settings using animal transplantation models, and these have provided comparatively good knowledge of the rejection reaction. Still, the relevance of the graft-versus-host reaction (GVHR) in small bowel transplantation has thus far remained doubtful. From a theoretic point of view, GVHR might play an important role in small bowel transplantation, because the small bowel graft contains a considerable number of immunocompetent cells in both the Peyer's patches and the mesenteric lymph nodes. These cells can be stimulated by host antigens and can attack the recipient immunologically [*1*].

The early results of experimental small bowel transplantation and the case reports of disastrous human small bowel grafting gave no clear evidence of GVHR [*2*]. Several investigators observed signs of GVHR after canine small bowel transplantation [*3–5*], whereas no GVHR could be demonstrated after small bowel transplantation in the pig model [*6,7*], and even recent reports give only an indistinct description of GVHR in small bowel transplantation [*8*].

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The reason for this indistinct description of GVHR-related phenomena lies in the undefined immunogenetic situation in allogeneic canine or pig transplantation. In these models, simultaneously occuring host-versus-graft reaction and GVHR interfere with each other; therefore, GVHR cannot express itself clearly. The first attempts to characterize GVHR in small bowel transplantation more exactly were made by Monchik and Russell [*9*] using parenteral strain donors and F_1 hybrid recipients from inbred rat strains, which thus made a unidirectional GVHR possible. These investigators were able to show distinct signs of GVHR which resembled those first described by Simonsen [*10*].

Since the first description of GVHR, its elucidation and prevention seems to be among the indispensible prerequisites for clinical small bowel transplantation. As a continuation of the aforementioned experiments, we carried out an investigation to describe GVHR and its underlying mechanisms in small bowel transplantation and tried to find methods of circumventing it.

Material and Methods

A semiallogeneic donor recipient combination was established by using Brown Norway ($RT1^n$) donor rats and Brown Norway- Lewis ($RT1^l$) F_1 hybrid recipients.

The operative procedure, which has been described in detail elsewhere [*11*], consists of accessory heterotopic transplantation of donor small bowel (Figure 1). The portal vein and the superior mesenteric artery of the graft are anastomosed to the recipient's inferior vena cava and

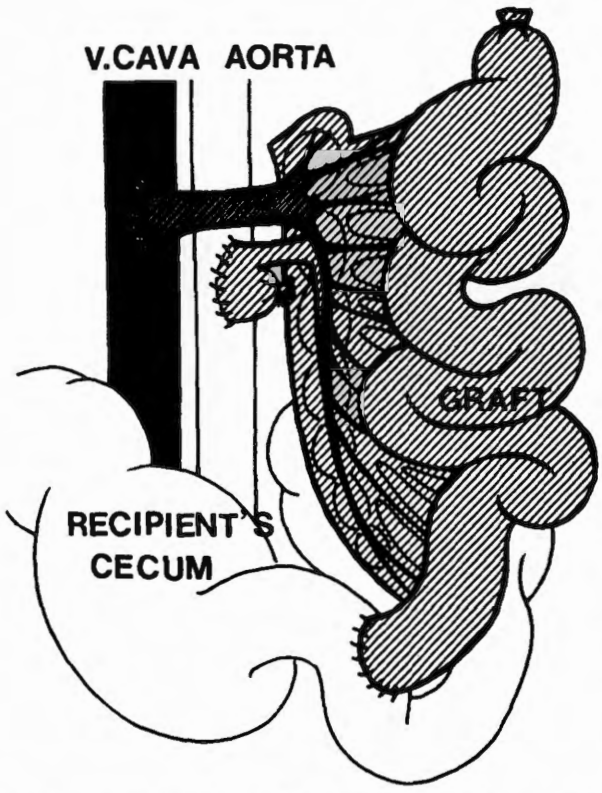

Figure 1. Transplantation of the heterotopic accessory small bowel.

TABLE I Experimental Groups

Group	Rats (n)	Treatment
1	26	None
2	28	Oral cyclosporine (15 mg/kg body weight 14 days postop)
3	26	Donor irradiation (950 rads)
4	26	Removal of mesenteric lymph nodes of the graft

Figure 2. Group 1 rat at autopsy 14 days postoperatively. Arrows to the left indicate the recipient's small bowel; arrow to the right indicates the graft.

TABLE II Survival Rates

Group	Treatment	n	%
1	No treatment	0/26	0
2	Cyclosporine	20/28	71
3	Donor irradiation	20/26	77
4	Removal of mesenteric lymph nodes	23/26	89

abdominal aorta, respectively. The oral end of the graft is closed and the distal end is anastomosed to the recipient's terminal ileum.

Four experimental groups were formed (Groups 1, 2, 3, and 4) (Table I). The 26 animals in Group 1 received no further treatment. The 28 animals in Group 2 received 15 mg of cyclosporine per kilogram body weight orally for 14 days, beginning with the first postoperative day. The 26 donors of Group 3 received irradiation consisting of 950 rads. The grafts were removed within 15 hours after irradiation. In Group 4 (26 animals), the mesenteric lymph nodes of the graft were removed microsurgically. This technique has been described elsewhere [*11*].

The cytotoxic antihost activity of T lymphocytes from different lymphatic compartments of the graft (namely, the mesenteric lymph nodes and Peyer's patches) and the recipient small bowel (namely, the mesenteric lymph nodes, Peyer's patches, peripheral cervical lymph nodes, and spleen) and in the recipient's blood was measured by a microcytotoxicity assay [*12*] using Lewis rat fibroblasts as target cells for determining GVHR activity. Specific lysis, which was determined by subtracting the cytotoxicity of syngeneically transplanted control animals from the cytotoxicity of semiallogeneic Groups 1 through 4, was expressed by the integral (cm^2) of a curve that resulted from the cytotoxicity values of different dilutions of T-cell suspensions. It is quoted as the mean ± standard error. The microcytotoxicity assay was performed at 14 days (Group 1), 20 to 30 days, and 82 to 120 days (Groups 2, 3 and 4) after transplantation. The statistical evaluation was carried out by means of the Wilcoxon-Mann-Whitney rank-sum test. Histologic examination of all the aforementioned tissues was performed with special attention to T-dependent areas of the lymph nodes and spleen.

Results

All animals in Group 1 died within 22 days after transplantation (Table II). Beginning with the 10th postoperative day, the animals lost weight, had diarrhea, and developed severe exfoliative dermatitis. As these symptoms became aggravated, the animals died. Autopsy revealed a characteristic pattern (Figure 2) with the graft intact. The mesenteric lymph nodes, however, were greatly enlarged (Figure 3). The recipient small bowel was thin walled

Figure 3. Group 1 rat. Semiallogeneic small bowel graft (arrow) 14 days after transplantation with enlarged mesenteric lymph nodes.

Figure 4. A semiallogeneic graft from a Group 1 rat 14 days postoperatively. All compartments of the bowel wall appear normal. (Giemsa stain; magnification × 160.)

and blown up with gas. Both the spleen (spleen index: 5.4 ± 3.3) and the peripheral lymph nodes were enlarged. Histologic examination of the graft (Figure 4) showed a normal bowel wall. The paracortical area of the mesenteric lymph nodes of the graft showed evidence of maximum immunologic stimulation in the form of proliferation of immunoblasts and macrophages (Figure 5). These signs reached their maximum expression at day 14 after transplantation and did not disappear until the death of the recipient. The small bowel of the recipi-

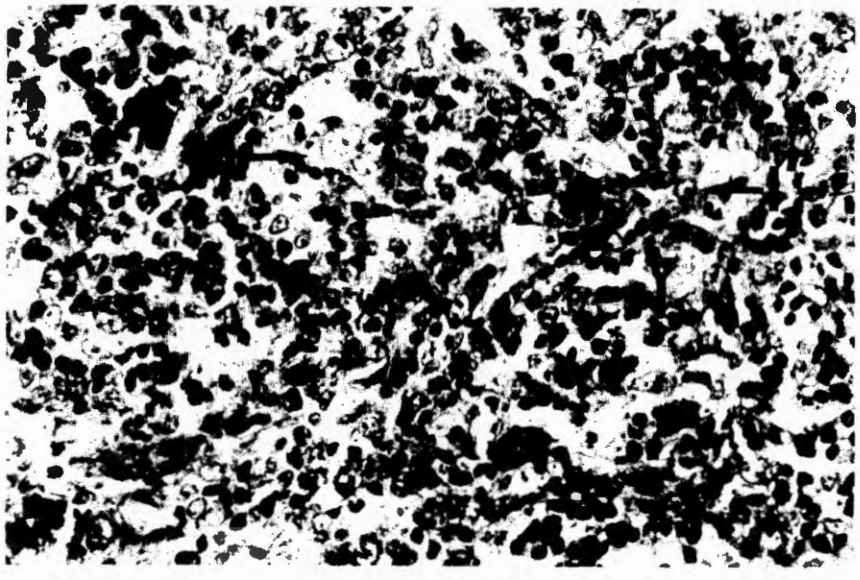

Figure 5. Group 1 rat. Paracortical area of mesenteric lymph nodes of the graft with proliferation of immunoblasts (I) and macrophages (M) 14 days postoperatively. (Giemsa stain; magnification × 400.)

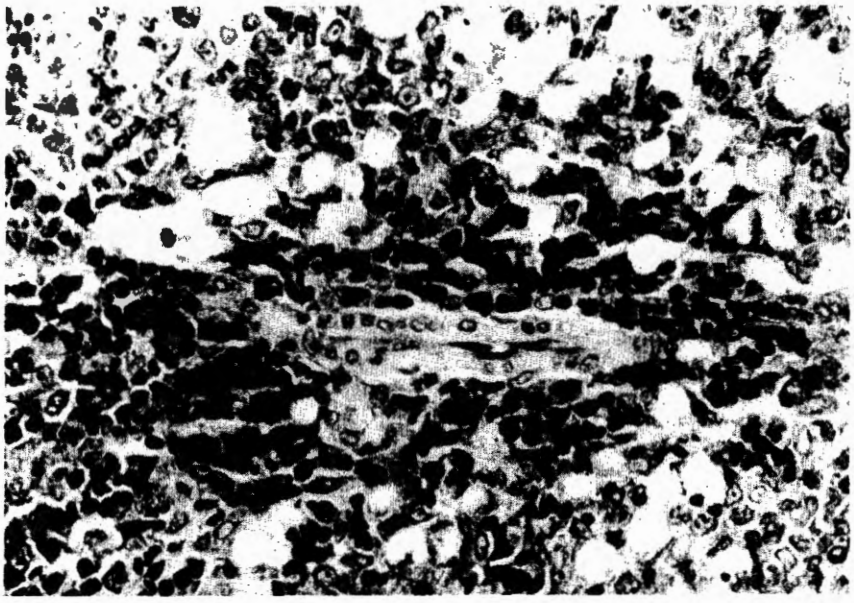

Figure 6. Spleen of recipient in Group 1 14 days postoperatively with proliferation of immunocompetent cells in the periarteriolar sheaths. (Giemsa stain; magnification × 400.)

ent showed signs of severe erosive enteritis with infiltration of the lamina propria by numerous immunoblasts and activated lymphatic cells that caused erosion of mucosal epithelium. Similar to the T-dependent areas of the graft's mesenteric lymph nodes, the paracortical areas of the peripheral lymph nodes and the periarteriolar sheaths of the recipient's spleen also showed strong immunologic stimulation in the form of proliferation of the immunocompetent cells (Figure 6).

The antihost T-cell cytotoxicity values in the microcytotoxicity assay (Figure 7, Table III) showed high GVHR activity in all lymphatic compartments except the Peyer's patches of the graft and the recipient. Extremely high graft versus host activity could be shown in the recipient's blood.

Twenty of 28 animals in Group 2 (71 percent) survived until 150 days after transplantation (Table II). Clinical signs of runting disease appeared only in two animals. Autopsy showed that the graft and the recipient small bowel were normal both macroscopically and histologically. No enlargement of the mesenteric lymph nodes of the graft could be shown. Histologic evaluation of the paracortical area of these lymph nodes revealed no immunologic stimulation either 20 or 110 days after transplantation. The peripheral lymph nodes of the recipient were not enlarged nor could a proliferation of immunologic stimulation in the periarteriolar sheath be shown. The recipient's spleen showed no immunologic stimulation in the periarteriolar sheath (Figure 8).

Antihost T-cell activity in Group 2 (Figure 7, Table III) had decreased to zero in the mesenteric lymph nodes of the graft 20 and 110 days after the operation. Graft versus host activity in the spleen had disminished significantly ($p < 0.01$) compared with Group 1 20 days postoperatively and reached zero 100 days after transplantation. The antihost activity found in the host Peyer's patches was not significantly different from that in Group 1 either 20 or 110 days postoperatively. No blood reactivity could be found. After irradiation of the donor animals (Group 3), 20 of 26 (77 percent) survived in good health until 120 days after transplantation. Twenty-three of 26 recipients (89 percent) of grafts with the mesenteric lymph nodes removed (Group 4) survived until 120 days after transplantation (Table II). Autopsy findings and histologic features of these two groups were similar.

The grafts and the small bowels of the recipients were normal, as was the histologic picture of the small bowel grafts. The mesenteric lymph nodes of the grafts in Group 3 showed no evidence of immunologic stimulation. The spleen and cervical lymph nodes of Group 3 and 4 animals revealed a normal histologic picture both 30 and 120 days postoperatively.

In Group 3, antihost cytotoxicity had decreased to zero (Table III, Figure 9) in the graft compartments, although on the 30th postoperative day, distinct T-cell reactivity could be found in the Peyer's patches and the spleens of the recipients. This was not significantly different from that of Group 1. Blood reactivity was zero on the 30th and 120th days. The same holds true for the reactivity in all other compartments. In Group 4, reactivity (Table III, Figure 9) had decreased to zero in all graft and donor compartments except the spleen during both periods of time. The spleen reactivity was, however,

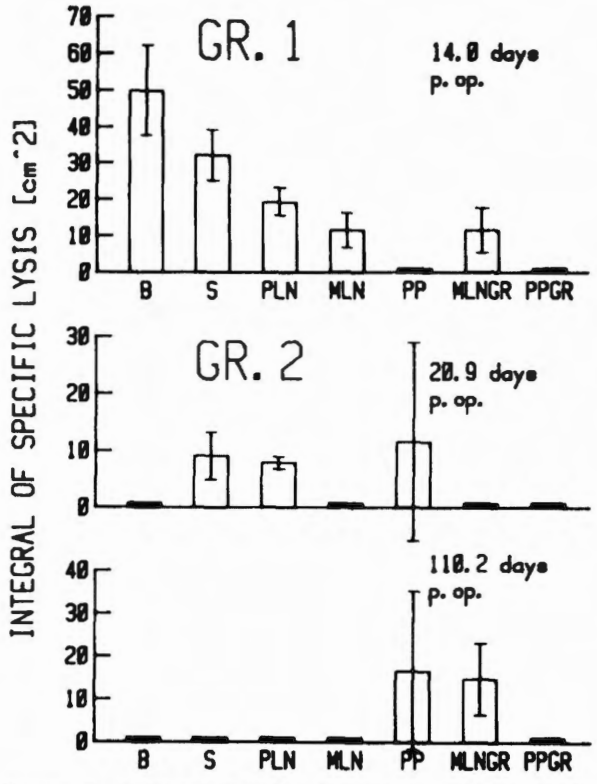

Figure 7. Antihost T-cell cytotoxicity values of the microcytotoxicity assay in Groups 1 and 2. The mean ± standard error of the mean are indicated. B = blood; MLN = mesenteric lymph nodes; MLNGR = mesenteric lymph nodes of graft; PLN = peripheral lymph nodes; PP = Peyer's patches; PPGR = Peyer's patches of graft; S = spleen.

significantly reduced ($p < 0.002$ at 29 days and $p < 0.05$ at 82 days) compared with Group 1.

Comments

After small bowel transplantation in all animals that had received a semiallogeneic small bowel graft without any treatment, a characteristic clinical picture developed that was identical to the runting syndrome that occurs in the course of GVHR, as described for the first time by Simonsen [*10*] after injection of immunocompetent cells. In the semiallogeneic donor-recipient combination chosen in our experimental model for immunogenetic reasons, an immunologic attack by donor cells against host antigens can develop without interference from HVGR. This experimental setting was chosen for the first time by Monchik and Russell [*9*] in their pioneering experiments on small bowel transplantation in the rat. The semiallogeneic small bowel transplantation in this experimental design revealed a clear-cut morphologic and functional pattern of GVHR. Aside from the skin, the small bowel of the recipient is a main target of antihost reactions [*13*]. The damage done to these epithelial organs by immunocompetent cells of the donor seems to be the main reason for the death of the recipients.

Lymphatic tissues of the grafts and recipients also show a very strong histologic alteration in the course of GVHR, thus demonstrating that lymphatic tissue is another main target of graft-versus-host reactions [*1*].

Immunologic stimulation of donor cells in the

TABLE III Antihost T-Cell Cytotoxicity Values of Microcytotoxicity Assay*

Group	No. Tested	Postop Day	B	S†	PLN	MLN	PP	MLNGR	PPGR
1									
14 d postop	11	14 ± 2.4	49.7 ± 12	32 ± 6.8	9.1 ± 3.5	11.5 ± 4.6	0	11.5 ± 5.9	0
2									
20.9 d postop	10	20 ± 7.2	0	9 ± 3.9	7.8 ± 0.9	0	11.5 ± 17.2	0	0
110.2 d postop	6	110.2 ± 18.7	0	0	0	0	16.5 ± 18.5	14.7 ± 8.1	0
3									
30.1 d postop	7	30.1 ± 0.9	0	27.8 ± 9	0	0	18 ± 5.5	0	0
120.6 d postop	10	120.6 ± 34.4	0	0	0	0	0	0	0
4									
28.7 d postop	7	28.7 ± 2.2	0	5 ± 1.2	0	0	0	. . .	0
82.2 d postop	9	82.2 ± 31.1	0	16 ± 7.5	0	4 ± 3.5	0	. . .	0

* Statistical values are the mean ± the standard error of the mean.

† $p < 0.01$ for the antihost T-cell cytotoxicity value in Group 2 20.9 days postoperatively versus that in Group 1; $p < 0.002$ for Group 4 28.7 days postoperatively versus that in Group 1; and $p < 0.05$ for Group 4 82.2 days postoperatively versus that in Group 1.

B = blood; MLN = mesenteric lymph nodes; MLNGR = mesenteric lymph nodes of graft; PLN = peripheral lymph nodes; PP = Peyer's patches; PPGR = Peyer's patches of graft; S = spleen.

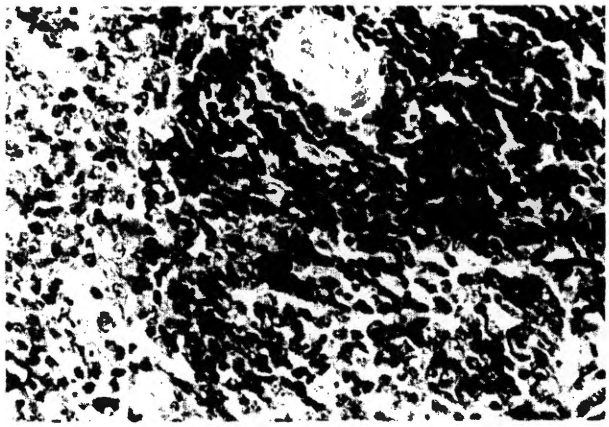

Figure 8. Normal appearance of the periarteriolar sheath of the recipient's spleen in Group 2 14 days postoperatively. (Giemsa stain; magnification × 400.)

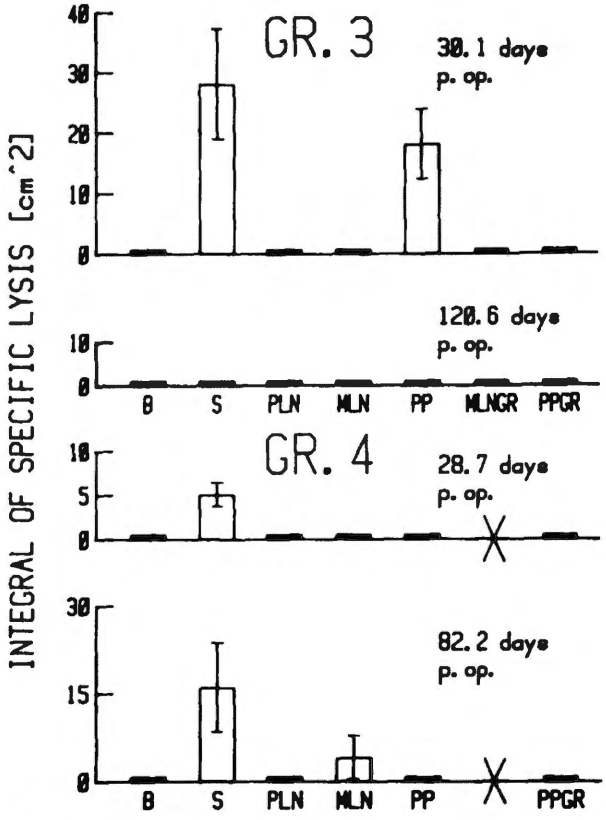

Figure 9. Antihost T-cell cytotoxicity values of the microcytotoxicity assay in Groups 3 and 4. The mean ± standard error of the mean are indicated.

macroscopically enlarged mesenteric lymph nodes of the graft, which can be seen histologically, represents the induction of GVHR in a donor compartment, whereas histologic alterations in the recipient's lymphatic tissues represent the continuing proliferation of donor cells in the recipient.

Functional data from the microcytotoxicity assay representing the effective activities of GVHR are in complete accordance with these morphologic findings, since strong histologic stimulation corresponds directly to distinct values of antihost cytotoxicity. The most outstanding feature of flourishing GVHR (Group 1) is the high rate of antihost cytotoxic activity in the blood of small bowel recipients.

For the expression of strong GVHR which leads to the death of the recipients (Group 1), a distinct antihost reactivity that is not locked into the lymphatic compartments and also appears at a high level within the blood seems to be characteristic. The decrease in this blood reactivity in the experimental groups after donor and recipient treatment (Groups 2, 3, and 4) accompanied by the marked increase in the survival rates (Table II) is the most remarkable result of these experiments. This finding underlines the significance of the antihost reactivity found in the recipient's blood for the effects of GVHR.

In the cyclosporine-treated Group 2, in addition to the decrease in this blood antihost activity, the antihost activity in the lymphatic compartments was found to be eliminated (Table III, Figure 7). The spleen reactivity 20 days after transplantation was significantly different from that in Group 1 ($p < 0.01$). Peripheral lymph node reactivity at 20 days postoperatively did not differ from the values in Group 1. The reactivity of the recipients' Peyer's patches, however, did not decrease. In contrast, it was higher than in Peyer's patches of Group 1 animals. One hundred ten days after transplantation, antihost reactivity in all compartments except the recipient's Peyer's patches disappeared, despite the fact that administration of cyclosporine ceased after 14 days.

The morphologic pattern in the T-dependent areas of the mesenteric lymph nodes of the graft represents the immunologic stimulation of the immunocompetent cells of the graft against donor antigen and the pattern in the T-dependent areas of the spleen of the recipient, their continuing proliferation within the recipient. Since these findings are absent after administration of cyclosporine, it can be concluded that cyclosporine is capable of suppressing the induction of antihost-directed immunologic stimulation of GVHR after small bowel transplantation. Not only the inductive phase of GVHR is influenced by cyclosporine, but also the antihost effector activities of T lymphocytes in the peripheral blood and the lymphatic compartments of the graft and recipient, thus causing avoidance of GVHR and survival of the recipients. This is shown by analysis of the functional parameters in the mi-

crocytotoxicity assay. Cyclosporine can thus suppress both the inductive phase of the antihost immune response (sensitization), as far as can be concluded from the histologic data, and the effector phase of GVHR, as is revealed by functional investigations. The decisive prerequisite for this graft-versus-host suppressing effect is the administration of cyclosporine immediately after small bowel transplantation during the sensitization period of GVHR. Once the development of GVHR has been hampered, cyclosporine can be discontinued.

The reason why an unaltered high antihost reactivity remains in the Peyer's patches is unclear. Since cyclosporine alters the lymphocyte migration patterns in cardiac allograft models [*14*], the increase in antihost activity in Peyer's patches in small bowel transplantation might be caused by alteration of lymphocyte migration behavior in cyclosporine-treated animals.

Sublethal irradiation of the donors also has a GVHR-suppressing effect. Animals survive and show no clinical signs of GVHR during the first 2 weeks after transplantation, which was the time of maximum expression of GVHR in the control group, although residual activity remains in the Peyer's patches and the spleen of the recipients 30 days after transplantation (Figure 9). This reactivity, however, decreases to zero 120 days postoperatively, thus proving irradiation to be effective for avoidance of GVHR over a long period of time.

The most impressive results in the direction of circumventing GVHR were seen after the removal of the graft's mesenteric lymph nodes (Table III, Figure 9). The remaining measurable antihost activity in the spleen was significantly lower than in Group 1 ($p < 0.002$, 28.7 days postoperatively and $p < 0.05$, 82.2 days postoperatively). These results show that the mesenteric lymph nodes of the graft are the tissue responsible for the development of GVHR. The T lymphocytes of the remaining Peyer's patches of the graft may develop antihost properties also, but these cells cannot elicit a GVHR that induces clinical symptoms.

With regard to the clinical applicability of these experimental methods for the suppression of GVHR, administration of cyclosporine seems appropriate for clinical use at the present time because it is already widely applied in transplantation. The methods involving irradiation of the graft and the removal of the mesenteric lymph nodes will have to be tested in experimental models of orthotopic nonauxiliary small bowel replacement. Alteration of the small bowel mucosa by irradiation or interruption of the lymphatic vessels by extirpation of the lymph nodes might impair the resorptive function of the small bowel graft. These experimental regimens of donor, graft, and recipient treatment have proved their efficacy for circumventing GVHR, and thus provide methods that are applicable in the clinical setting.

Summary

To describe GVHR in small bowel transplantation and its underlying mechanisms and to find methods for circumventing that response, accessory small bowel transplantation was carried out in the rat model. Animals not treated with cyclosporine, irradiation, or removal of the mesenteric lymph nodes of the graft died within 22 days postoperatively due to graft versus host disease. Mesenteric lymph nodes of the graft and recipient spleen and peripheral lymph nodes showed strong immunologic stimulation histologically and high antihost T-cell-mediated cytotoxic antihost reactivity. Seventy-one percent of the animals that had received 15 mg of cyclosporine per kilogram body weight orally survived 150 days after transplantation. After donor irradiation with 50 rads, 77 percent of the recipients survived 120 days. After microsurgical removal of the mesenteric lymph nodes of the graft, 89 percent survived 120 days.

We conclude that GVHR plays an important role in small bowel transplantation and that the experimental regimens of donor, graft, and recipient treatment described herein have proved their efficacy for circumventing GVHR.

References

1. Gleichmann E, Rolink AG, Pals ST, Gleichmann H. Graft versus-host reactions (GVHRs): clues to the pathogenesis of a broad spectrum of immunologic diseases. Transplant Proc 1983;15:1436–40.
2. Hardy MA, Iga C, Lau H. Intestinal transplantation: laboratory experience and clinical consequences. In: Thiede A, Deltz E, Engemann R, Hamelmann H, eds. Microsurgical models in rats for transplantation research. New York: Springer Verlag, 1985.
3. Lillehei RC, Idezuki Y, Feemster JA, et al. Transplantation of the stomach, intestine, and pancreas: experimental and clinical observations. Surgery 1967;62:721–41.
4. Alican F, Hardy JD, Cayirli M, et al. Intestinal transplantation: laboratory experience and report of a clinical case. Am J Surg 1971;121:150–9.
5. Cohen B, MacGregor AB, Moore KTH, Falk RE, Langer B, Cullen JB. Canine small bowel transplantation; a study of immunological responses. Arch Surg 1976;111:248–53.
6. Hay JM, Fagniez PL, Parc R, et al. Results of orthotopic homotransplantation of the small bowel in the pig. Ann Chir 1974;28:1063–70.
7. Stauffer UG, Becker M, Mona D, Kara D. Transplantation of the small intestine; an experimental study in young minipigs. Res Exp Med 1978;172:161–71.
8. Craddock GN, Nordgren SR, Reznick RK, et al. Small bowel transplantation in the dog using cyclosporine. Transplantation 1983;35:284–8.
9. Monchik GJ, Russell PS. Transplantation of small bowel in the rat: technical and immunological considerations. Surgery 1971;70:693–702.
10. Simonsen M. Graft versus host reactions. Their natural history and applicability as tools of research. Progr Allergy 1962; 6:349–467.

11. Deltz E, Thiede A. Experimental transplantation of small intestine. Microsurgical techniques and their applicability in research. In: Olszewsky W, ed. Handbook of microsurgery. Vol. 2. Boca Raton, FL: CRC Press, 1984:419–29.
12. Bröcker EB, Kuhlencord KM, Müller-Ruchholtz W. Microcytotoxicity test in allograft immunity: specificity and kinetics of effector T cells. Int Arch Allergy Appl Immunol 1977;53: 234–41.
13. Deltz E, Müller-Hermelink HK, Ulrichs K, Thiede A, Müller-Ruchholtz W. Development of graft-versus-host reaction in various target organs after small intestine transplantation. Transplant Proc 1981;13:1215–6.
14. Kuromoto N, Iga C, Fawwaz R, Nowygrod R, Reemtsma K, Hardy MA. Effect of cyclosporine on lymphocyte migration in rat cardiac transplantation. Transplant Proc 1983;14 (suppl 1):2973–8.